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ARMY ARMAMENT RESEARCH AND DEVELOPMENT COMMAND ABERD--ETC F/G 6/6  
THE TOXICITY OF PHOSPY WATER TO SELECTED FRESHWATER ORGANISMS.(U)

MAR 78 J G PEARSON, P F ROBINSON, E S BENDER

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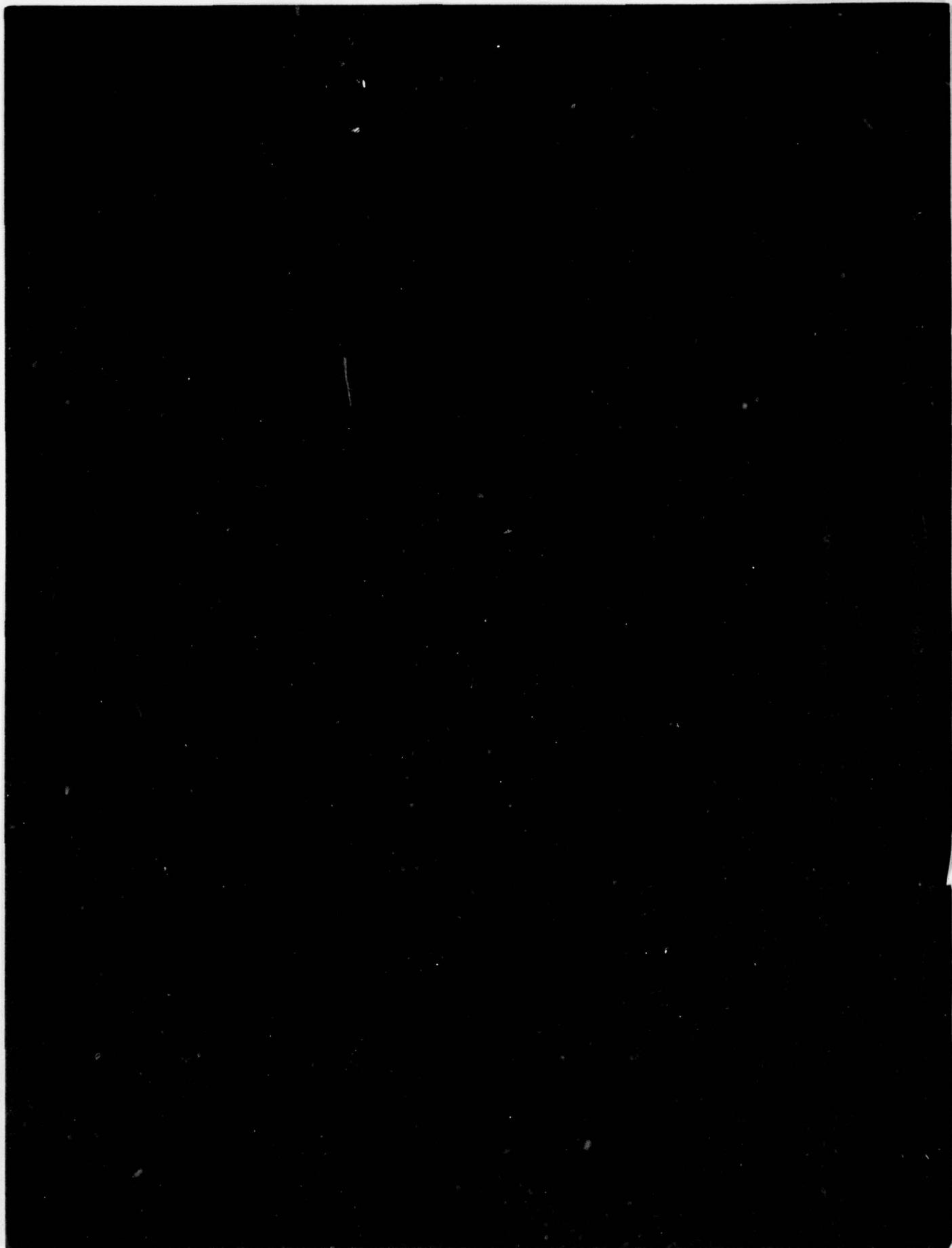


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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) (U) Six species of freshwater organisms were exposed to the wastewater from a white phosphorus munitions filling facility, Pine Bluff Arsenal, Pine Bluff, Arkansas, in stated toxicity tests. Both fish ( <i>Gambusia affinis</i> and <i>Lepomis macrochirus</i> ) and benthic macroinvertebrates ( <i>Glyptotendipes sp.</i> , <i>Palaemonetes kadiakensis</i> , <i>Chaoborus punctipennis</i> , and <i>Branchiura sowerbyi</i> ), endemic to the area, were tested for periods of up to 96 hours. The median effective concentration (EC50) for all the invertebrates was between the LC50 for the bluegill ( <i>L. macrochirus</i> , 29.0 µg/l P4) and the mosquito fish ( <i>G. affinis</i> , 75 µg/l P4). MICROFILM		

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PREFACE

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## TOXICITY OF PHOSSY WATER TO SELECTED FRESHWATER ORGANISMS

### I. INTRODUCTION.

During pollution surveys at Pine Bluff Arsenal (PBA), Pine Bluff, Arkansas,<sup>1,2</sup> it was found that the discharge from the white phosphorus munitions filling plant was having an adverse impact on the receiving creek and further downstream on Yellow Lake. The discharge from this facility (known as phossy water) contains substantial amounts of elemental phosphorus ( $P_4$ ). Elemental phosphorus has been shown to be extremely toxic to marine fish<sup>3</sup> (lethal at 1.0  $\mu\text{g/l}$ ) and to significantly accumulate in the livers of cod (25,000 times ambient).<sup>4</sup> Only Isom<sup>5</sup> has reported any toxicity data on freshwater organisms, namely the bluegill.

Because of the lack of information on the toxicity of  $P_4$  to freshwater organisms and the known occurrence of  $P_4$  in the discharge, aquatic toxicity tests were conducted on freshwater organisms endemic to PBA in November 1974. This report describes the results of those tests.

### II. METHODS.

#### A. Chemical.

Elemental phosphorus determinations were made by gas-liquid chromatography with a flame photometric detector specific for  $P_4$ .<sup>6</sup> All phossy water samples (50 ml) were extracted with benzene and the extract was immediately frozen. Using this method, the detection limit was approximately 0.5 ppb, and reproducible results were obtained at approximately 1.0 ppb. Water-quality determinations were made according to standard methods.<sup>7</sup>

#### B. Aquatic Toxicity Tests.

Organisms used for testing were collected from the Yellow Lake area with the exception of bluegills which were obtained from a local fish hatchery. Species collection sites are given below. (See figure for site location in Yellow Lake.)

<u>Species</u>	<u>Location</u>
<i>Gambusia affinis</i>	Below old spillway
<i>Lepomis macrochirus</i>	Local fish hatchery
<i>Palaemonetes kadiakensis</i>	Yellow Lake (near eastern shore)
<i>Glyptotendipes</i> sp.	Yellow Lake 4
<i>Chaoborus punctipennis</i>	Yellow Lake 1
<i>Branchiura sowerbyi</i>	Yellow Lake 1

All test animals were maintained in the laboratory for at least 24 hours before they were tested. Yellow Lake water from station 11 was used for holding water and also for dilution water during the toxicity tests. The only exception to the above was the water used for bluegills. The bluegills were held in dechlorinated tap water for at least 2 weeks prior to being tested; this water was also used as the dilution water during the bluegill toxicity tests.

Both species of fish were tested in glass jars containing 15 liters of water (15 to 20 fish/container). All invertebrate species were tested in glass dishes containing 1.0 liter of water (5 organisms/container).

Because of the reactive nature of the P<sub>4</sub> in the phossy water, all tests were set up immediately after the phossy water discharge sample was taken. Just prior to initiating the fish toxicity test, 50 ml samples from each concentration were taken for P<sub>4</sub> analysis. P<sub>4</sub> concentrations in the invertebrate test were based on calculated values from a known solution of phossy water.

Preliminary tests were conducted on all six species to establish the order of magnitude of toxicity of the phossy water. Final tests were conducted on three species: *G. affinis*, *L. macrochirus*, and *P. kadiakensis*.

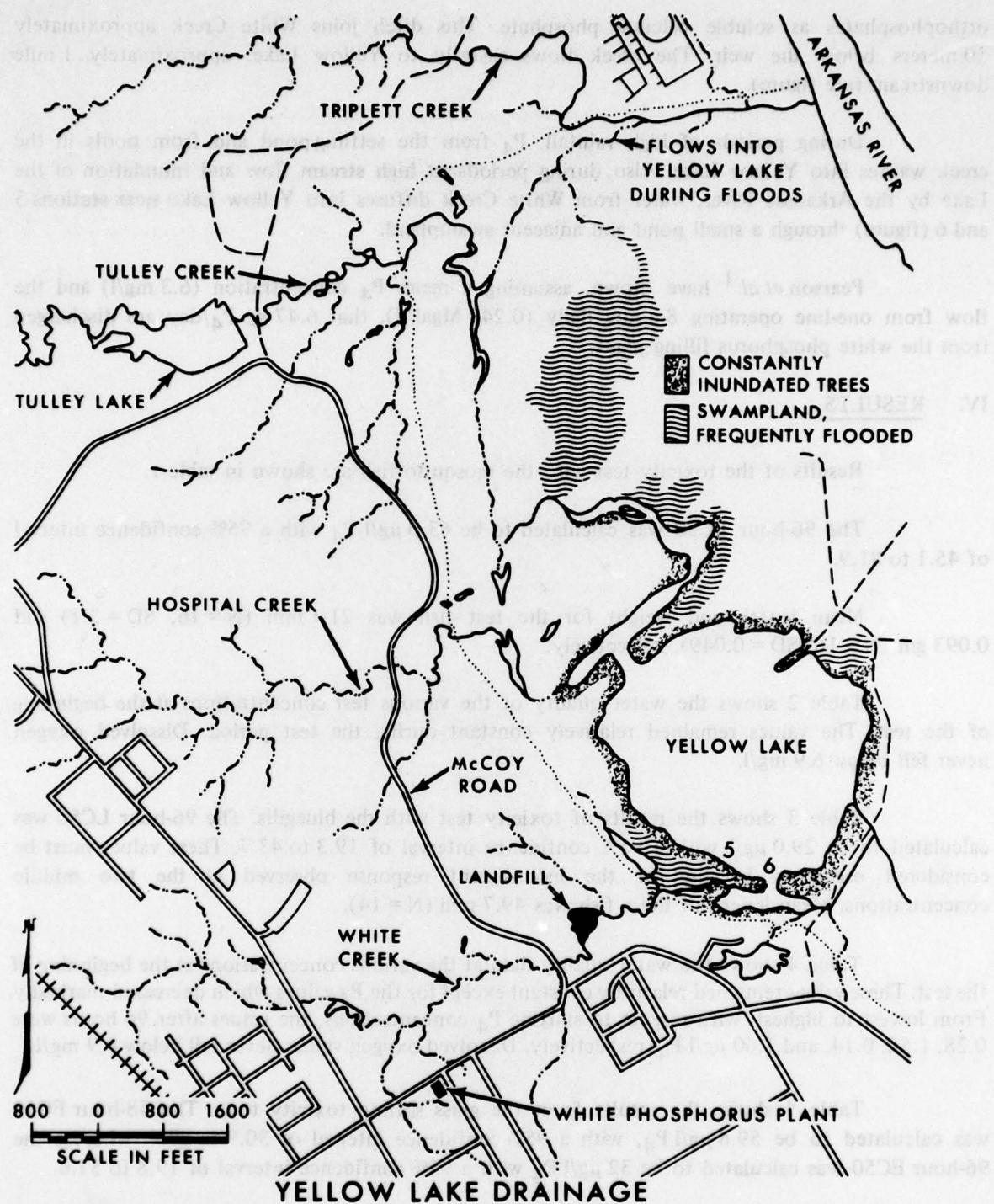
#### C. Data Analysis.

Median effect (EC50's and LC50's) calculations were made according to the maximum likelihood method of Finney.<sup>8</sup> A computerized probit analysis was used to calculate the values of a and b in the equation Y = a + bX. "X" represents the toxicant concentration and Y is the normal deviate of the proportion of fish dying at the concentration, with the value of 5.0 added to eliminate the possibility of "Y" being negative. After a and b are calculated, the median effect value can be easily derived. The proportion of 50% mortality corresponds to a normal deviate of 0 and, therefore, a Y value of 5, so the median effect value is simply the X for which a + bX = 5. The 95% confidence interval was computed for each median effect value according to Bliss.<sup>9</sup> In those cases where only one partial kill was obtained, the median effect value and its confidence interval were calculated according to the moving average angle method of Harris.<sup>10</sup>

In some toxicity tests, the response at the lower concentration was zero mortality, and the response at the next higher concentration was 100% mortality. In such "all-or-nothing" cases, a probit analysis could not be performed. In these cases, the median effect concentrations were estimated by taking the square root of the product of the two consecutive concentrations between which there was a jump from zero to 100% mortality. These two concentrations were also used as estimates of a confidence interval about the median effect concentration.

#### III. CHARACTERISTICS OF THE PHOSSY WATER DISCHARGE.

The wastewater from the white phosphorus filling plant is piped underground northwesterly to a small settling pond. The overflow from this pond flows into an open ditch through a V-notch weir. The upper 10 meters of the ditch contains limestone to precipitate the



**Figure. Phossy Water Discharge Site and Receiving Water**

orthophosphates as soluble calcium phosphate. This ditch joins White Creek approximately 50 meters below the weir. The creek flows easterly to Yellow Lake, approximately 1 mile downstream (see figure).

During periods of high rainfall,  $P_4$  from the settling pond and from pools in the creek washes into Yellow Lake. Also, during periods of high stream flow and inundation of the Lake by the Arkansas River, water from White Creek diffuses into Yellow Lake near stations 5 and 6 (figure) through a small pond and adjacent swampland.

Pearson *et al.*<sup>1</sup> have shown, assuming a mean  $P_4$  concentration (6.3 mg/l) and the flow from one-line operating 8 hours daily (0.243 Mgal/d), that 6.47 kg  $P_4$ /day are discharged from the white phosphorus filling plant.

#### IV. RESULTS.

Results of the toxicity test with the mosquito fish are shown in table 1.

The 96-hour LC50 was calculated to be 63.0  $\mu\text{g/l}$   $P_4$  with a 95% confidence interval of 45.1 to 81.9.

Mean length and weight for the test fish was 21.1 mm ( $N = 16$ ,  $SD = 3.1$ ) and 0.093 gm ( $N = 16$ ,  $SD = 0.049$ ), respectively.

Table 2 shows the water quality of the various test concentrations at the beginning of the test. The values remained relatively constant during the test period. Dissolved oxygen never fell below 6.9 mg/l.

Table 3 shows the results of toxicity test with the bluegills. The 96-hour LC50 was calculated to be 29.0  $\mu\text{g/l}$  with a 95% confidence interval of 19.3 to 43.7. These values must be considered estimates because of the inconsistent response observed at the two middle concentrations. Mean length of these fish was 49.7 mm ( $N = 14$ ).

Table 4 shows the water quality data at the various concentrations at the beginning of the test. These values remained relatively constant except for the  $P_4$  values which decreased markedly. From lowest to highest, with respect to starting  $P_4$  concentrations, the values after 96 hours were 0.28, 1.58, 0.14, and 1.60  $\mu\text{g/l}$   $P_4$ , respectively. Dissolved oxygen values never fell below 6.9 mg/l.

Table 5 shows the results from the grass shrimp toxicity tests. The 48-hour EC50 was calculated to be 59.6  $\mu\text{g/l}$   $P_4$ , with a 95% confidence interval of 30.9 to 88.4; whereas the 96-hour EC50 was calculated to be 32  $\mu\text{g/l}$   $P_4$  with a 95% confidence interval of 19.8 to 51.6.

Table 6 shows the water quality data at the various concentrations at the beginning of this toxicity test. As before, these values remained relatively constant, with the probable exception of  $P_4$ .

Table 1. Number of Mosquito Fish (*Gambusia affinis*) Surviving  
Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (μg/l)				
	Control	21	73	192	220
hr					
0	20	20	20	20	20
24	20	20	15	1	0
48	20	20	9	0	0
72	20	20	6	0	0
96	20	20	6	0	0

Table 2. Water Quality at the Beginning of the Mosquito Fish Toxicity Test

P <sub>4</sub>	Dissolved oxygen	Temperature	pH	Conductivity	Alkalinity (mg/l as CaCO <sub>3</sub> )	Hardness (mg/l as CaCO <sub>3</sub> )
μg/l	mg/l	°C		μmhos/cm <sup>2</sup>		
0	7.5	20.5	7.60	190	65	50
21	7.4	20.5	7.50	195	60	60
73	7.3	20.5	7.29	200	55	55
192	6.9	20.5	6.96	210	55	40
220	6.7	20.5	6.75	220	50	25

Table 3. Number of Bluegills (*Lepomis macrochirus*) Surviving  
Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (μg/l)				
	Control	1.2	11	34	60
hr					
0	15	15	15	15	15
24	15	15	11	15	14
48	15	15	11	15	1
72	15	15	11	15	0
95	15	15	11	12	0

Table 4. Water Quality at the Beginning of the Bluegill Toxicity Test

P <sub>4</sub>	Dissolved oxygen	Temperature	pH	Conductivity	Alkalinity (mg/l as CaCO <sub>3</sub> )	Hardness (mg/l as CaCO <sub>3</sub> )
μg/l	mg/l	°C		μmhos/cm <sup>2</sup>		
0	8.95	20.25	7.72	190	60	50
1.2	8.70	20.5	7.68	170	65	55
11	8.75	20.5	7.65	180	65	60
34	8.75	20.5	7.55	180	60	50
60	8.70	19.95	7.55	120	60	60

Table 5. Number of Grass Shrimp (*Palaemonetes kadiakensis*)  
Surviving Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (μg/l)				
	Control	21	73	192	220
hr					
0	12	13	11	11	14
24	12	13	11	4	4
48	12	11	4	0	0
72	12	9	2	0	0
96	12	9	2	0	0

Table 6. Water Quality at the Beginning of the Invertebrate Toxicity Tests

P <sub>4</sub>	Dissolved oxygen	Temperature	pH	Conductivity	Alkalinity (mg/l as CaCO <sub>3</sub> )	Hardness (mg/l as CaCO <sub>3</sub> )
μg/l	mg/l	°C		μmhos/cm <sup>2</sup>		
0	8.9	21.0	8.35	305	70	70
21	8.8	21.0	8.30	300	65	75
73	8.8	21.0	8.20	288	70	75
192	8.9	21.0	8.10	285	65	70
220	8.6	21.0	7.95	280	60	70

Tables 7, 8 and 9 show the results of preliminary toxicity tests with *B. sowerbyi*, *Glyptotendipes* sp. and *C. punctipennis*, respectively. These data indicate that the 48-hour EC50's for these organisms are somewhere between 12 and 120 µg/l P<sub>4</sub>. It was estimated to be 37.9 µg/l P<sub>4</sub> for all species (see methods for details of the calculations).

Table 7. Number of Sludge Worms (*Branchiura sowerbyi*)  
Surviving Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (µg/l)*			
	Control	1.2	12	120
hr				
0	5	5	5	5
24	5	5	5	5
48	5	5	5	1

\* Nominal concentrations: based on a solution of phossy water containing 120 µg/l P<sub>4</sub>.

Table 8. Number of Midge Larvae (*Glyptotendipes* sp.)  
Surviving Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (µg/l)*			
	Control	1.2	12	120
hr				
0	5	5	5	5
24	4	4	4	0
48	4	4	4	0

\* Nominal concentrations: based on a solution of phossy water containing 120 µg/l P<sub>4</sub>.

Table 9. Number of Phantom Midge Larvae (*Chaoborus punctipennis*)  
Surviving Exposure to Phossy Water

Time	Concentration P <sub>4</sub> (μg/l)*			
	Control	1.2	12	120
hr				
0	5	5	5	5
24	4	5	5	3
4	5	5	5	0

\* Nominal concentrations: based on a solution of phossy water containing 120 μg/l P<sub>4</sub>.

#### V. DISCUSSION AND CONCLUSIONS.

The results from all of the toxicity tests are summarized in table 10. Review of this table shows that phossy water is extremely toxic as a result of its P<sub>4</sub> content. Bentley<sup>11</sup> has shown the 96-hour LC50 of pure P<sub>4</sub> ranges from 6 to 73 μg/l for fish tested under laboratory conditions. He has also shown that the 48-hour EC50 ranges from 30 to 560 μg/l for invertebrate species tested under the same conditions. The data in table 10 show good agreement with these values, again indicating the P<sub>4</sub> is responsible for the toxicity observed in phossy water.

Table 10. Summary of Aquatic Toxicology Results

Species	Median effect concentration	95% Confidence interval
<i>Gambusia affinis</i>	75.0 <sup>a</sup>	66.1–85.1
<i>Lepomis macrochirus</i>	29.0 <sup>a</sup>	19.3–43.7
<i>Palaeomonetes kadiakensis</i>	59.6 <sup>b</sup>	30.9–88.4
<i>Glyptotendipes</i> sp.	37.9 <sup>c</sup>	12.0–120.0 <sup>c</sup>
<i>Chaoborus punctipennis</i>	37.9 <sup>c</sup>	12.0–120.0 <sup>c</sup>
<i>Branchuria sowerbyi</i>	37.9 <sup>c</sup>	12.0–120.0 <sup>c</sup>

<sup>a</sup> 96-Hour LC50.

<sup>b</sup> 48-Hour EC50.

<sup>c</sup> "All-or-nothing" responses (see method for details of the calculations).

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